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EVALUATION OF VARIOUS OILS ON KHAPRA BEETLE, *TROGODERMA GRANARIUM* EVERTS (COLEOPTERA: DERMESTIDAE) IN TERMS OF SURVIVAL OF ADULTHOOD, GRAIN DAMAGE AND POPULATION BUILD-UP

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ABSTRACT

Khapra beetle (*Trogoderma granarium* Everts) is a serious pest of stored grains and having a status of a dangerous quarantine pest for many a country. Its detection attracts serious economic and trade restrictions. It is presently controllable only by methyl bromide (CH_3Br), which is facing a blanket ban in many countries due to its ozone depleting nature. Hence an alternate management strategy is envisaged by using eco-friendly, economic, effective, non-hazardous, easily available and easy to use material. A part of this study relates to the usage of materials that are user-friendly and do not demand any sophisticated equipments. Present paper evaluates the six commonly available oils that are non-hazardous, eco-friendly and can be used in a sustainable and economic way in containing the menace of Khapra beetle.

KEYWORDS: Khapra Beetle (*Trogoderma Granarium* Everts), Ozone Depletion, Grain-Coating, Edible / Non-Edible Oils, Ecofriendly

INTRODUCTION

The Khapra beetle, *Trogoderma granarium* Everts is one of the most notorious primary insect pests of stored grains (Banks, 1977; Hill, 1983). It causes direct and various indirect losses (El Nadi *et al.*, 2001). It is a very serious pest under hot dry conditions. If infestation is severe, the devastation is complete, reducing the grain to mere frass (EPPO, 1990). Its exuviae, shed skin and other body parts are carcinogenic to human beings. It is a polyphagous and most feared upon pest, from quarantine point of view, especially in western countries that are of strategic importance to India for exports of cereals, pulses, oilseeds, etc. Presence of this pest attracts trade restriction implications.Non-Khapra beetle countries enforce quarantine restrictions on the imported commodities from Khapra beetle countries. The US Government spent about $15 million for its eradication programme, when it was accidentally introduced into USA (Kerr, 1981). In India, many export shipments have suffered heavy losses due to detection of this pest in one or other form. Russia banned imports of plant products from India owing to detection of this pest in a consignment of sesame (Reuters, 2006; HT Media, 2007). Administration of Quality Supervision, Inspection and Quarantine (AQSIQ) Authority of China returned 460 tons of soybeans imported from the Scoular Company, USA due to detection of Khapra beetles, despite the consignment being certified by the Quarantine Authority of US.

Protection of stored grains from insect damage is currently dependent mainly on synthetic pesticides such as fumigation with phosphine or methyl bromide or dusting with compounds like primiphos-methyl and permethrin (Price and Mills, 1988; Singh, 1990). The wide spread use of such chemicals has significant drawbacks i.e., development of strains

resistant to pesticides (Zettler and Cuperus, 1990), increased costs, handling hazards, insecticide residues on grains and great threat to environment and human health. Outbreaks of environmental hazards related to contribution of fumigants such as methyl bromide on the degradation of stratospheric ozone (Taylor, 1994) initiated calls to phase out methyl bromide usage.

A study conducted under laboratory conditions was planned to develop an alternate management protocol in the scheme of IPM, which would be nature friendly, effective, economical, safe, sustainable and easy to apply, for the control of the important primary insect pest of stored grains, *i. e*. Khapra beetle. Present paper describes the effect of three edible and three non-edible oils, by way of their coating on wheat grains, on the Khapra beetle.

MATERIALS AND METHODS

The insects were maintained in round glass jars of 1 Kg capacity, half filled with whole wheat grains and their mouths covered with double folded muslin cloth held tight with the help of 4˝ rubber bands around its neck. The wheat was properly dried, cleaned and conditioned. The culture was allowed to breed for three generations. The controlled conditions maintained in the laboratory were 27±1˚C temperature, 65±5 % relative humidity and 12 h photoperiod.

The oils used in these experiments are as follows -

Edible Oils

- Coconut oil @ 1% (v/w).
- Soybean oil @ 1% (v/w).
- Mustard oil @ 1% (v/w).

Non–Edible Oils

- Neem Oil @ 1% (v/w).
- Pongamia (Karanj) oil @ 1% (v/w).
- Camphor oil @ 0.5% (v/w).

The oils were procured in local market from reputed shops. Preliminary studies were conducted to arrive at the proper doses of the oils to be used. Three doses *i.e.* 0.2% (v/w) 0.5% (v/w) and 1% (v/w) of the oils were tested and an effective dose in each particular case was selected for detailed studies.

One Kg of the grain conditioned as per the earlier description was taken in each of the round glass jars of 2 Kg capacity. All these jars have smooth inner surface. The required concentration of the oil or the pesticide was prepared by diluting in 20 ml of the solvent acetone. Then the preparation was added to the grain and the lid of the bottle was tightened. The control jar received only 20 ml acetone. Then the jars were shook vigorously to achieve the uniform mixture. Then the lids were removed and the solvent was allowed to evaporate completely under ceiling fan. The jars were intermittently subjected to vibration. After 30 minutes, they were covered with double folded muslin cloth held tight with 4˝ rubber bands. They were properly labeled. All these jars served as stock material for further experimentation.

From among the above stock, 50 gram grain / 1000 grains - as per the requirement - were taken from each of the treated jar and placed into a small plastic jar. 5 pairs of freshly emerged adults / 30 third instar larvae of Khapra beetle

were released in small jars. Then, the jars were covered with muslin cloth as usual. Three replicates were maintained.

Observations were taken after one month. The various parameters of the observation were as under-

- Effect on survival to adulthood [By using larvae].
- Effect on grain damage [By using larvae].
- Effect on population build-up [By using adults (males & females)].

50 grams of grains were used in parameter (1) and (3) above, while for parameter (2) above, 1000 grains were used. Similarly, such experiments were conducted upto 9 months, once every month, using the treated stock grain and by taking 5 pairs of freshly emerged adults / 30 third instar larvae.

These experiments were planned to assess the efficacy of the oils in protecting the grain from the test insect in terms of their effect on adults / larvae / kernel damage over a period of time which is 9 months in the present studies.

Statistical Analysis

The statistical analysis done by the one way (ANOVA) and Post Hoc tests were performed for multiple comparisons for all the three parameters i.e. survival of adulthood, percent grain damage and population build- up. The graph was prepared in spreadsheets of Microsoft Excel (2010). All the results are tabulated, statistically processed and presented.

RESULTS

LARVAL EXPERIMENTS

Survival to Adulthood

Among non-edible oils; Pongamia oil was observed to be superior over neem oil followed by camphor oil in an overall context. Pongamia oil offered complete protection upto six months, Neem oil for four months and Camphor oil for two months. In this period there was 100 % mortality of the introduced larval population. Pongamia and neem oils lost their effect gradually with time, though they showed some residual effect even after 9 months. However, Camphor oil rapidly lost its effect after 2 months. All treatments were superior to Control.

Among edible oils, as an overview of the period under study, soybean oil was superior to mustard oil followed by coconut oil in arresting the survival of the introduced larval population to the adulthood. All three edible oils caused 100% mortality to introduced larvae upto 2 months. The effectiveness of all the treatments was inversely proportional to the time and a gradual decrease in the mortality of introduced larval population was observed with the progress of time. All treatments were superior to Control in which there was cent percent survival of the introduced larval population. The detailed data regarding survival to adulthood is given in Table 1. The statistical analysis is performed using one way ANOVA & Post Hoc test (Table 2).

The effect of oils (Edible & non- edible) on percent survival of *Trogoderma granarium* Evert on adulthood were represented in (Figure 1 & 2).

Percent Grain Damage

Among non-edible oils; Grain Damage recorded was directly proportional to time in all of the cases. Surprisingly,

Camphor oil recorded sharp increase in damage percentage and even caused more damage than in control. Pongamia oil offered complete protection upto 6 months, whereas neem oil and camphor oil protected grains fully upto 4 and 2 months respectively. The graph showed percent grain damage by *Trogoderma granarium* Evert (Figure 3)

Among Edible oils; Soyabean oil proved effective over all oils. All treatment was superior over control for most of the period of study. All treatment gave complete protection from the *Trogoderma granarium* upto a period of 2 months & thereafter the damage was directly proportional to time. The percent grain damage was presented in (Figure 4)

ADULT EXPERIMENTS

Population Build-Up

Among non-edible oils; in Pongamia oil treatment, absolutely no population build up to the period of study was noticed. Neem Oil prevented population build up to 6 months. However, the build up after 6 months was less, when compared with control. In case of Camphor oil treatment, its effect was seen decreased from month-3 onwards and the population went on increasing gradually. Surprisingly, the population build up was more than control at the end of eighth month and it was far more at the end of nine months. (Figure 5)

Among edible oils; Soybean oil totally prevented the beetle from building up its population up to 4 months, whereas coconut oil and mustard oil were successful on this count up to 3 months. Thereafter; i.e. 5 month onwards in soybean oil and 4 month onwards in other two, population build up steadily increased. This increase was more or less proportional and similar to each other in soybean and mustard oils. However, in the case of soybean oil, the build-up was rather slow and it remained less than 50% compared to control at the end of 9 month period. (Figure 6)

The graphical representations of the results obtained are produced in Figure 1 & 2 (survival to adulthood), 3 & 4 (percent grain damage) and 5 & 6 (population build-up) for edible and non- edible oils.

DISCUSSIONS

In present studies different oils, *i.e.* Coconut, Mustard, Soybean, Neem and Pongamia @ 1% (v/w) and Camphor oil @ 0.5% (v/w) were used as grain protectants for nine months. Generally speaking, all the oils were effective at varying period of time in causing mortality and checking the grain damage and the population build-up.

It appears, that among non-edible oils, Pongamia oil was more toxic followed by Neem oil and then by Camphor oil. Among edible oils Soybean was more toxic to the introduced larval population followed by Mustard and Coconut oils which appeared *at par* among themselves. In all cases the toxicity decreased with time.

The oils of Pongamia followed by Neem were observed to be repellent / antifeedant for longer period *i.e.* for six and four months respectively. The effect gradually reduced thereafter. In case of Camphor oil, it appears that the complete protection for first two months is due to its suffocating / fumigating action rather than direct larval toxicity. This hypothesis gains strength as the grubs fed on the grains lavishly after the initial two month period and later the damage caused was greater than the damage in Control. This is in contrast with other oils which showed repellent / antifeedant effect though it was gradually decreased. This phenomenon shows that Camphor oil, instead of repellency, has in fact acted as an attractant, probably by virtue of some stimulus present in it.

Among edible oils, all the three oils used showed the repellency and / or antifeedant property which was retained uniformly upto the end of two months and later the effect was gradually decreased. The soybean oil was superior on this

count followed by coconut oil followed by mustard oil.

The population build-up in the present studies indicates the growth disrupting properties of the material used. Pongamia oil treated grain did not allow any population build-up and it remained NIL upto the end of nine months. No population build-up took place for six month in Neem oil. The reason for the inhibition of population build-up could be due to the growth regulating and gonadotropic effect of the compounds used on the adult / larval stages of the test insect, thereby resulting in a low oviposition and affecting subsequent hatchability.

Camphor oil, interestingly has acted differently. No larva survived upto two months but the survival was *at par* with control from 5th month onwards. As far as the grain damage was concerned, it gave total protection upto the end of two months; however, it lost its effect rapidly so much so that the damage recorded was equivalent to or more than that of in control from the end of 6th month. The population surprisingly surpassed the Control populations from 8th month onwards.

These observations indicate that the initial desirable effect on survival of grubs, grain damage and population build-up was due to the suffocating / fumigating properties of the camphor oil. This effect is lost in a later period as the camphor oil is evaporative in nature. It exhibited the phagostimulant action and has shown the attracting properties instead of repellency.

Camphor oil has been reported effective by Garcia (1990), Qiantai and Yongcheng (2001) and Liu *et al.* (2005) on other stored pests. The results in the present study are only in partial agreement with other works as the toxicity due to camphor oil was noticed in the present experiment, but the factor of repellency to the test insect is not well exhibited. On the contrary, it has acted as an attractant. The studies of Padin *et al.* (1999) reported similar results with camphor oil while conducting the studies on the repellency factor on blowflies.

Among edible oils; no population build up was noticed for the period of four months in case of Soybean oil and for three months in case of Coconut as well as in Mustard oil. This is mainly attributed to the toxicity to grubs. The build-up increased gradually thereafter in all treatments indicating reduction in the residual toxicity with passage of time.

Applicability of the usage of oils in protecting storage grains to supplement safe chemical formulations has gained momentum of late. The mode/s of action, appropriate dosages and duration of efficacy of oils has been investigated by various workers on different storage pests (Singh *et al*., 1978). The amount of oil needed for the control of most storage pests varied from 2 cc/kg seed to 15 cc/kg seed (Cruz and Cardona, 1981) depending on the level of infestation.

The presence of so-called secondary compounds, which have no known function in photosynthesis, growth or other aspects of plant physiology, give plant materials or their extracts their anti-insect activity. Secondary compounds include alkaloids, terpenoids, phenolics, flavonoids, chromenes and other minor chemicals. They can affect insects in several ways: they may disrupt major metabolic pathways and cause rapid death, act as attractants, deterrents, phagostimulants, antifeedants or modify oviposition. They may retard or accelerate development or interfere with the life cycle of the insect in other ways (Bell, *et al.*, 1990).

In addition to action against adult insects, vegetable oils are generally reported to exert ovicidal action (Don-Pedro, 1989a). It was suggested that egg mortality was caused by the physical properties of the oil coating, blocking respiration, rather than by a specific chemical effect.

Akou-Edi (1985) reported that because of the antifeedant and repellent activities of neem oil, red corn seeds were protected from confused flour beetles and corn weevils. Sunitha (2006) reported that neem oil reduced the population and disrupted growth and development of *Acanthoscelides obtectus, Sitophilus oryzae, Oryzaephilus surinamensis and Cryptolestes ferrugineus.* Neem seed oil's larval growth inhibitor, antifeedant and moult disrupting activities were measured by Isman *et al.* (1990) against insect pests. Mustard, Pongamia and neem oils also possibly exert ovicidal action. It is proposed that the oils are acting as mimics of the marker substance of the test insect thereby reducing drastically the egg deposition by *Callosobruchus chinensis* (Singh and Yadava, 2003). Singh *et al.* (1994) reported that oils of soybean, coconut, mustard, among others, caused reduced adult emergence and delayed the developmental period of *Callosobruchus chinensis*.

The results obtained in present study are also in agreement with the findings of the above workers. Thus, the different oils are used in the present investigation against *Trogoderma granarium* Evert and evaluated on the parameters of mortality, grain damage and population build-up. It is possible that the oils are directly toxic to the grubs. They also have some ovicidal action.

The present theme of studies thus revolves around the importance of food grain storage, havoc caused by storage pests, the given notorious status of *Trogoderma granarium* Everts among them, difficulty in its control and necessity to have an alternate management protocol in the scheme of Integrated Pest Management.

The use of locally available plants avoids the need to establish complex mechanisms for pesticide distribution; the community can collect or grow the plants itself. The use of plant materials for storage protection is sustainable, can be continuously propagated year after year, biodegradable and do not have any negative impact on the environment.

Integrated Pest Management (IPM) is today's *Mantra* of Plant Protection. The usage of plant oils in the manner evaluated shall form an important and integral protocol in the holistic scheme of IPM as the plant oils used in the present studies are safe, non-hazardous, eco-friendly, easily available, handy, easy to use, economical and sustainable besides being found effective.

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APPENDICES

Table 1: Effect of Oils on Percent Survival of *Trogoderma granarium* Everts to Adulthood (*30 Grubs per Treatment, per Replication)*

Time/ Treatment	Non-Edible Oils			Edible Oils			Control
	Camphor Oil	Pongamia Oil	Neem Oil	Soybean Oil	Coconut Oil	Mustard Oil	
01 month	NIL	NIL	NIL	Nil	Nil	Nil	100
02 months	NIL	NIL	NIL	Nil	Nil	Nil	100
03 months	46.67	NIL	NIL	13.33	33.33	37.00	100
04 month	84.67	NIL	NIL	23.67	48.67	48.67	100
05 months	100	NIL	40.00	32.67	59.67	58.33	100
06 months	100	NIL	49.67	40.00	72.67	73.00	100
07 months	100	53.67	60.00	53.33	82.77	83.33	100
08 months	100	66.67	70.67	63.33	90.00	90.00	100
09 months	100	70.00	82.33	70.00	100	100	100

Table 2: Statistical Analysis Using One Way ANOVA, Post – Hoc Test and Multiple Comparison were Performed for Survival to Adulthood1 & Survival of Adulthood 2

Descriptives

survival to adulthood1

	N	Mean	Std. Deviation	Std. Error
Camphor Oil	9	21.0000	13.00000	4.33333
Pongamia Oil	9	6.3333	9.59166	3.19722
Neem Oil	9	10.1111	10.24017	3.41339
Control	9	30.0000	.00000	.00000
Total	36	16.8611	13.13915	2.18986

Descriptives

survival to adulthood2

	N	Mean	Std. Deviation	Std. Error
Soybean	9	9.8889	7.80135	2.60045
Coconut	9	16.3333	11.10180	3.70060
Mustard	9	16.3333	11.02270	3.67423
Control	9	30.0000	.00000	.00000
Total	36	18.1389	11.18967	1.86495

ANOVA

survival to adulthood1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3115.417	3	1038.472	11.354	.000
Within Groups	2926.889	32	91.465		
Total	6042.306	35			

ANOVA

survival to adulthood2

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1937.417	3	645.806	8.453	.000
Within Groups	2444.889	32	76.403		
Total	4382.306	35			

Multiple Comparisons

Dependent Variable: survival to adulthood1
LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.
Camphor Oil	Pongamia Oil	14.66667*	4.50839	.003
	Neem Oil	10.88889*	4.50839	.022
	Control	-9.00000	4.50839	.054
Pongamia Oil	Camphor Oil	-14.66667*	4.50839	.003
	Neem Oil	-3.77778	4.50839	.408
	Control	-23.66667*	4.50839	.000
Neem Oil	Camphor Oil	-10.88889*	4.50839	.022
	Pongamia Oil	3.77778	4.50839	.408
	Control	-19.88889*	4.50839	.000
Control	Camphor Oil	9.00000	4.50839	.054
	Pongamia Oil	23.66667*	4.50839	.000
	Neem Oil	19.88889*	4.50839	.000

*. The mean difference is significant at the .05 level.

Multiple Comparisons

Dependent Variable: survival to adulthood2
LSD

(I) treatment	(J) treatment	Mean Difference (I-J)	Std. Error	Sig.
Soybean	Coconut	-6.44444	4.12048	.128
	Mustard	-6.44444	4.12048	.128
	Control	-20.11111*	4.12048	.000
Coconut	Soybean	6.44444	4.12048	.128
	Mustard	.00000	4.12048	1.000
	Control	-13.66667*	4.12048	.002
Mustard	Soybean	6.44444	4.12048	.128
	Coconut	.00000	4.12048	1.000
	Control	-13.66667*	4.12048	.002
Control	Soybean	20.11111*	4.12048	.000
	Coconut	13.66667*	4.12048	.002
	Mustard	13.66667*	4.12048	.002

*. The mean difference is significant at the .05 level.

(A) Survival of Adulthood

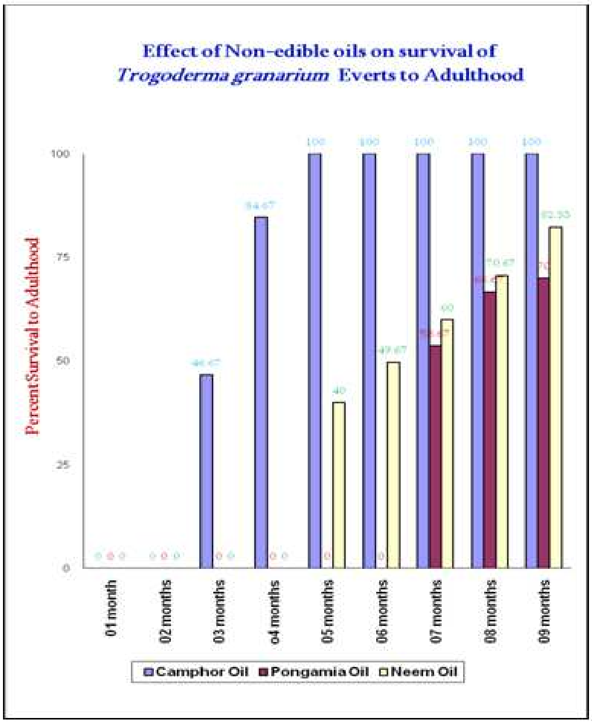

Figure 1: Effect of Non- Edible Oils on Survival of *Trogoderma granarium* Everts to Adulthood

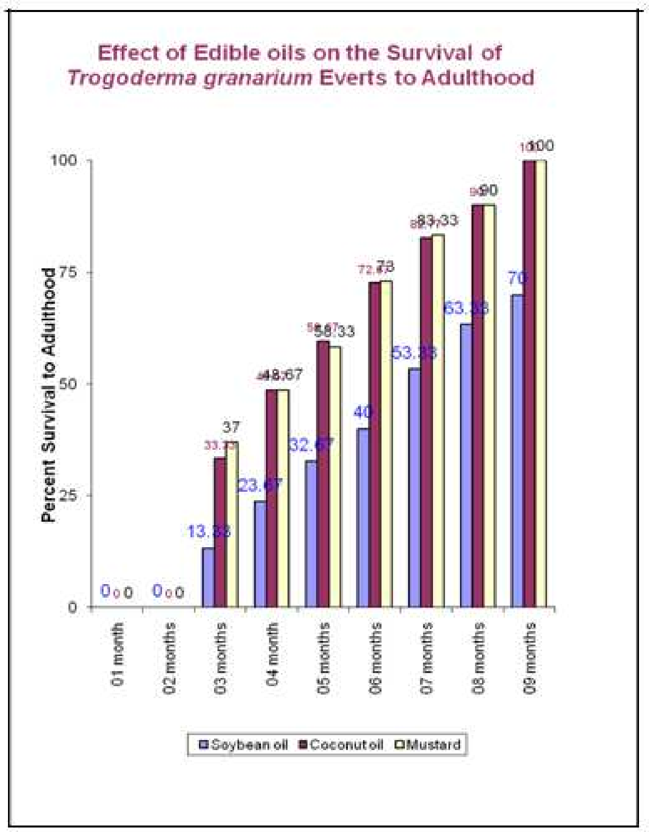

Figure 2: Effect of Edible Oils on the Survival of *Trogoderma granarium* Everts to Adulthood

(B) Percentage Grain Damage

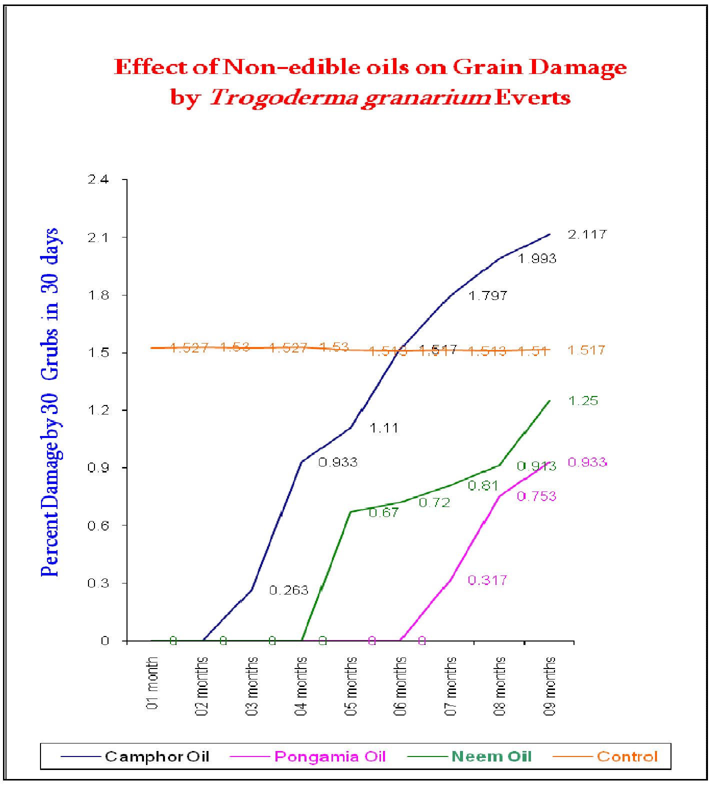

Figure 3: Effect of Non- Edible Oils on Grain Damage by *Trogoderma granarium* Everts

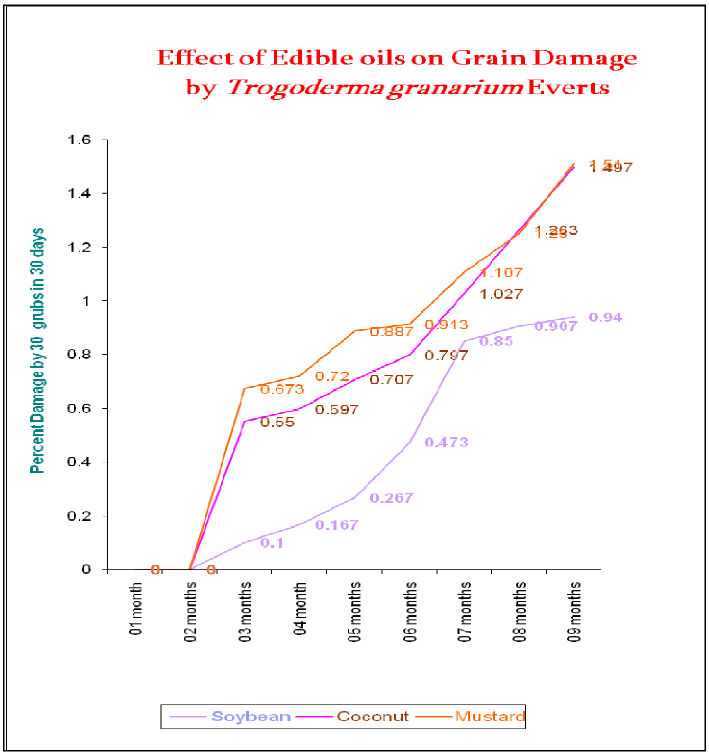

Figure 4: Effect of Edible Oils on Grain Damage by *Trogoderma granarium* Everts

(C) Population Build-up

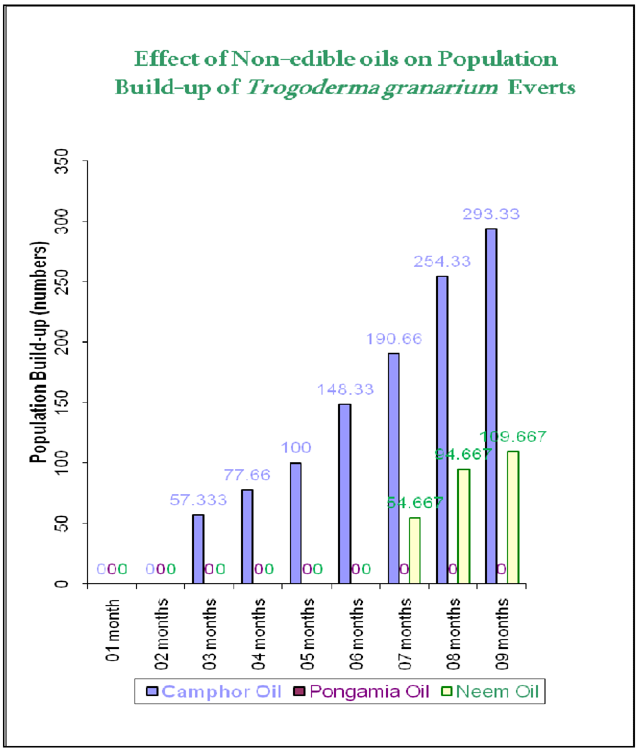

Figure 5: Effect of Non- Edible Oils on Population Build- up of *Trogoderma granarium* Everts

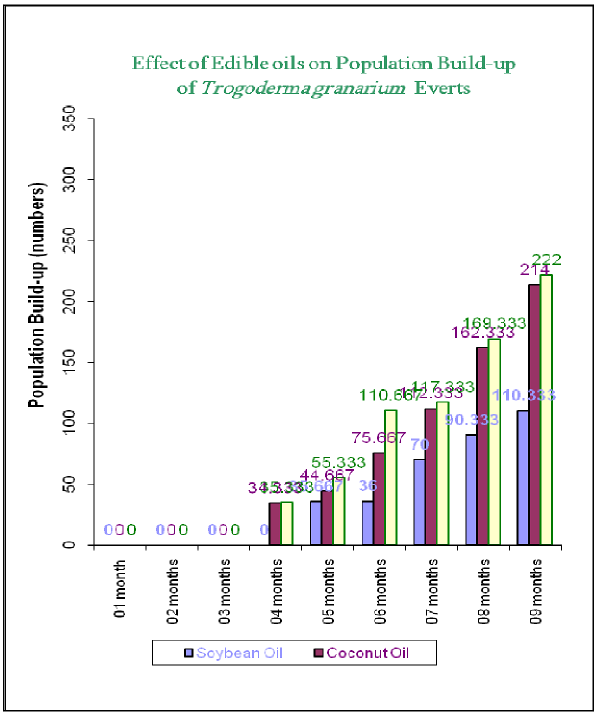

Figure 6: Effect of Edible Oils on Population Build – up of *Trogoderma granarium* Evert